INTERVIEW SUMMARY

Applicant's Representative thanks Examiner Larry R. Helms for the opportunity to interview this application by telephone on January 15, 2002. Issues relating to 35 U.S.C. § 112 and 35 U.S.C. § 103 were discussed during the interview, as were proposed amendments to the claims. Applicant has amended claims 1 and 5 in accordance with the Examiner's suggestion.

REMARKS

The present invention relates to "artificial mini-antibodies" or "monobodies," *i.e.*, polypeptides made of Fn3 β-strand domains and one or more loop regions, where the monobodies are capable of binding to a variety of specific binding partners.

Claims 1, 2, 4 and 5 are amended; claim 3 is canceled; as a result, claims 1, 2 and 4-6 are now pending in this application. No new subject matter has been added. The cancellations and amendments have been made to clarify the claims in order to expedite prosecution of the present application, and not for reasons of patentability. Therefore, the amendments are not intended to limit the scope of equivalents to which any claim element may be entitled. The amendments to the claims are fully supported by the specification as originally filed.

Claim 1 has been amended to recite that at least one monobody loop region sequence has been varied as compared to the wild-type loop region sequence, where the loop region is linked between two Fn3 β-strand domain sequences. The phrase "at least two" and "plurality" synonymous in common patent usage. *See, e.g.*, Landis on Mechanics of Patent Claim Drafting (4th ed., PLI 1996) at page III-17 ("plurality . . . used for an indefinite number, two or more") (copy attached). Applicant is following this general practice of having "at least two" and "plurality" be synonymous. Support for the recitation of "at least two," therefore, is found throughout the specification, for example at page 6, lines 20 and 21; page 7, lines 8, 9, 19, 20 and 30; page 8, lines 6 and 7; page 9, lines 7-9; page 10, lines 18-20; and original claims 1, 11-15, 31 and 33.

Claim 1 has also been amended to recite that the variation in the loop(s) can be by deletion of at least two amino acids in the loop region sequence, insertion of at least two to 25 amino acids, or replacement of at least two amino acids in the loop region sequence. The word

"or" is used herein in conformance with common patent law practice in the conjunctive or disjunctive, *i.e.*, and/or. Support for the deletion of "at least two amino acids" is found in originally filed claims 1 and 5. Support for the replacement of "at least two amino acids" is also found in originally filed claims 1 and 5. The Examiner's attention is also drawn, for example, to Table 1 on page 21 and Table 5 on page 48 of the specification where many different combinations of deletions and/or replacements are depicted. It is, therefore, well within the ability of one having skill in the art, in conjunction with the teachings of the application, to make many different loop variants. Support for the insertion of "at least two" is found in originally filed claims 1 and 5, and support for "25 amino acids" is found in claim 6.

Claim 1 has also been amended to recite that the polypeptide monobody of the present invention binds to a specific binding partner (SBP) to form a polypeptide:SBP complex. Support for this amendment is found in claim 2, and throughout the specification (*e.g.*, page 28, line 18 through page 30, line 18; page 32, line 15 through page 33, line 25; page 43, line 7 through page 46, line 28; page 47 line 15 through page 49, line 28; and page 53, line 26 through page 57, line 20).

Claims 2, 4 and 5 have been amended so as to have proper antecedent basis in claim 1. These amendments are being made for formal reasons only, and therefore do not limit the scope of equivalents to which the claims are entitled.

Information Disclosure Statement

The Examiner states that the Holm reference cited in the IDS filed 7/24/01 (Paper No. 19) was not previously provided. Enclosed is an Information Disclosure Statement which includes the full citation and a copy of this reference. **Applicant requests that the Examiner initial and return the attached Form 1449.**

§112 Rejection of the Claims

Claims 1-6 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled commensurate in scope with the specification. Claim 1 has been amended; insofar as the rejection is applied to the pending claims, it is hereby traversed.

Claim 1 has been amended to recite certain structural and functional elements of the monobody. In particular, at least one monobody loop region sequence varies (as compared to the wild-type loop region sequence) by deletion of at least two amino acids in the loop region sequence, insertion of at least two to 25 amino acids, or replacement of at least two amino acids in the loop region sequence. Further, the polypeptide monobody binds to a specific binding partner (SBP) to form a polypeptide:SBP complex.

To the extent that the Examiner's statement is intended as support for an argument that claim 1 encompasses inoperative embodiments, the Examiner is requested to note that claims are in accord with the requirements of 35 U.S.C. § 112 if one of skill in the art, guided by the specification, could avoid inoperable combinations and practice the invention without undue experimentation. The mere possibility that a claim embraces inoperable embodiments does not render it unduly broad. In addition, it is not a function of the claims to specifically exclude all possible inoperative substances.

It should be noted that the present claims are not directed to a monobody that necessarily has the same protein folding/conformation as that of native Fn3, or a portion of Fn3 if not all of the beta-strands are present. Instead, the claims recite a polypeptide monobody that has a certain structure (at least two Fn3 β-strand domain sequences with a loop region sequence linked between each Fn3 β-strand domain sequence, where the loop region has been modified by an insertion, deletion and/or replacement) and a certain function (binds to a specific binding partner (SBP) to form a polypeptide:SBP complex). The specification teaches appropriate tests to determine if the resulting monobodies have the claimed function of being able to bind to an SBP. *See, e.g.,* page 28, line 18 through page 30, line 18; page 32, line 15 through page 33, line 25; page 43, line 7 through page 46, line 28; page 47 line 15 through page 49, line 28; and page 53, line 26 through page 57, line 20. Compliance with 35 U.S.C. §112 must be adjudged from the perspective that claims are addressed to a person of average skill in the particular art, who would not choose a combination that would render a claimed composition inoperative. *Ex Parte Cole,* 223 U.S.P.Q. 94 at 95-96 (Bd. Pat. App. 1983) (copy enclosed). One skilled in the art in possession of the present specification could readily ascertain whether a compound comprising a given loop variation would be suitable for a certain SBP. Accordingly, the Examiner is

respectfully requested to find the pending claims to be in compliance with 35 U.S.C. §112, first paragraph.

The January 22, 2001 Office Action at page 4, lines 1-2 states that the claims are not enabled because at least two Fn3 beta-strand domains would not be as stable as the entire Fn3 domain. The Examiner may, in fact, be correct that this smaller molecule may not be as stable. The claims, however, do not require such stability, but simply that the molecule has sufficient structure so as to give it the functionality of being able to bind to a specific binding partner (SBP) to form a polypeptide:SBP complex.

The Office Action states that one skilled in the art would be forced into undue experimentation in order to practice the broadly claimed invention. Applicant asserts that the present patent specification teaches one skilled in the art how to make and use the full scope of the claimed invention without undue experimentation. The scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art. The present specification teaches the starting material for the monobody (*i.e.*, at least two Fn3 β-strand domain sequences with a modified loop region sequence linked between each Fn3 β-strand domain sequence), and that the resulting polypeptide monobody binds to an SBP. Thus, the specification is enabling.

If the Examiner takes the view that the specification must not only indicate the starting material, but also teach how the modification in the loop region is to occur (*e.g.*, substitution, deletion, or insertion), then Applicant maintain that the specification is still enabling. Some experimentation would be needed in order to test all the possible new proteins that could be made and be covered by Applicant's application. The amount of experimentation, however, would not be undue in view of teaching of the specification. The factors to be considered under *In re Wands*, 858 F.2d 731, 8 U.S.P.Q.2d (BNA) 1400, 1404 (Fed. Cir. 1988) in determining whether a disclosure would require undue experimentation include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

The specification provides a significant amount of direction and guidance (**factor 2**), and that guidance is in the form of actual working examples (**factor 3**). Example VI teaches methods for making loop variegations in the FG and BC loop. Examples IX, X, XII, and XVI teach the selection of phage-displayed monobodies that bind to target molecules.

Additional experiments were performed by the inventor that further support the pending claims. Declaration of Shohei Koide at ¶¶ 2-4. Mutant FNfn10 proteins that contain glycine insertions or glycine-rich insertions in the AB, BC, CD, DE, EF or FG loops were prepared and were functional. Also, non-glycine mutations were made in the AB loop that were functional in binding to their SBP. Declaration of Shohei Koide at ¶¶ 5-6.

The skill of those in the art (**factor 6**) is quite high in the fields of molecular biology and immunology, as evidenced by the level of sophistication of the experiments set forth in the specification. The state of the prior art (**factor 5**) with respect to what was known about Fn3 was well-developed.

Regarding the quantity of experimentation necessary (**factor 1**) and the predictability or unpredictability of the art (**factor 7**), mathematically a large number of monobody molecules could be generated and screened. With respect to "undue experimentation," the fact that the outcome of a synthesis/screening program is unpredictable is precisely why a screening program is carried out. The Office simply cannot reasonably contend that a screening program to locate biomolecules with target biological properties would not be carried out by the art worker because the results cannot be fully predicted in advance. In fact, the Federal Circuit has explicitly recognized that a need to carry out extensive synthesis and screening programs to locate bioactive molecules does not constitute undue experimentation. *In re Wands*, 8 U.S.P.Q.2d 1400, 1406-1407 (Fed. Cir. 1988). In *Wands* the court held that a process of immunizing animals, fusing lymphocytes from the immunized animals with myeloma cells to make hybridomas, cloning the hybridomas, and screening the antibodies produced by the hybridomas for the desired characteristics did not require undue experimentation. The Court stated:

> The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics.

> Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody.

Likewise, practitioners having skill in the art related to the present application, given the teachings of the present specification, would be well-equipped to prepare and screen polypeptide monobodies that bind to a specific binding partner. Thus, the fact that a claim may encompass a large number of polypeptide monobodies is not dispositive of the enablement issue. This is particularly true in an art area in which the level of skill is very high, and where the specification gives working examples on how to make various polypeptide monobodies (Examples I through VIII), and how to test a monobody's ability to bind to an SBP (Examples IX through XIV).

Considering all eight of the *Wands* factors, it clearly would not require undue experimentation to obtain polypeptide monobodies commensurate in scope with the pending claims. Appellants therefore assert that the specification fully enables one skilled in the art to construct polypeptide monobodies made of Fn3 β-strand domains and one or more loop regions, where the monobodies are capable of binding to a variety of specific binding partners.

The Examiner further states that claim 3 is not enabled. Applicant disagrees with the Examiner, but has cancelled claim 3 in order to expedite prosecution.

Applicant, therefore, requests that the rejections under 35 U.S.C. § 112 be withdrawn.

§103 Rejection of the Claims

Claims 1-6 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Main *et al.* (Cell 71:671-678, 1992, IDS #5) and further in view of Lee *et al.* (Protein Engineering 6:745-754, 1993, IDS #8).

Applicant continues to assert that the examiner has not established a *prima facie* case of obviousness. In order to establish a *prima facie* cases of obviousness, three factors must be met. First, the references themselves must teach or suggest all the limitations of the claims. Second, there must be a reasonable expectation of success at the time the invention was made. Third, the prior art must contain some suggestion or incentive that would have motivated the skilled artisan to modify a reference, or to combine references. Applicant respectfully asserts that the examiner has not met these three requirements for the pending claims.

The examiner cites Main *et al.* This reference discusses in detail the three-dimensional structure of the tenth type III module of fibronectin and compares it to several other protein structures, including the folding structure of immunoglobulin domains. "In this paper we describe the overall tertiary fold and dynamic properties of this module and the characteristics of the RGD motif it contains." (page 671, col. 2) At page 674, col. 2 of the reference, the authors state that the topology of fibronectin is similar to that of immunoglobulin C domains. (*See* also, Fig. 5 of Main *et al.*) The authors of the article were particularly interested in the binding of fibronectin to its integrin receptors, such as by means of RGD sequences that may be present in various loop regions. They proposed that the loops bearing RGD sequences may be flexible, rather than existing in a specific conformation (p. 676, col. 1). This conformational flexibility allows fibronectin to bind to its integrin receptors, even if its affinity and specificity is modulated in nature as a consequence of events within the cell. Nowhere in Main *et al.* is the possibility discussed that various loop regions be modified at the amino acid sequence level, *i.e.*, that there be structural changes introduced into the fibronectin molecule. As discussed above, it states that conformational changes occur in the molecule in response to a cellular environment, but it does not discuss artificially introduced sequence modifications.

In the initial Office Action, the Examiner quoted the last sentence of the "Results and Discussion" section on p. 676 as providing support for replacing amino acids in a fibronectin molecule ("The structure presented in this paper gives insight into the way a functional loop can be built onto a structural framework and, by virtue of its flexibility, be able to perform a wide range of functions.") This sentence, however, must not be taken out of context. The focus of the paper is on the conformational structure of a full-length, native fibronectin molecule. The full paragraph in which the cited sentence is found states the following:

> The binding of fibronectin to its integrin receptors is further complicated by the fact that integrin affinity and specificity can be modulated as a consequence of events withing cells (Hynes, 1992). Clearly, the interaction between fibronectin and its receptors can be finely tuned to encompass the wide range of integrin interactions among different cells. The structure presented in this paper gives insight into the way a functional loop can be built onto a structural framework and, by virtue of its flexibility, be able to perform a wide range of functions.

When read in the context of the whole reference, and in particular the specific paragraph in which it is found, Applicant asserts that this sentence merely summarizes that the natural RGD loop as found in the native fibronectin molecule has a great deal of flexibility, and performs a wide range of functions. Nowhere in the paper does the paper extrapolate the term "built" to mean a structurally modified sequence as was done by the present inventor. Also, the term "flexible" is used throughout the paper as meaning conformational flexibility, not inter-changeability of particular amino acids in the protein structure.

Thus, Main *et al.* do not teach or suggest that loop regions can be structurally changed by means of substitution, deletion or insertion of amino acids in various loop regions without detrimental effects on functionality of the molecule. Therefore, all the elements of the claims are not taught by Main *et al.* Further, there is no suggestion or motivation to modify Main *et al.* to develop the claimed invention, since Main *et al.*, as discussed above, does not make any suggestion that the loop regions could or should be modified. Again, Main *et al.* discuss the conformational flexibility of fibronectin, and not sequence "flexibility."

Lee *et al.* do not remedy the deficiencies of Main *et al.* Lee *et al.* discuss the design, construction and binding analysis of a series of mutants in which an RGD sequence has been inserted into two specific proteins of known sequence, namely the immunoglobulin light chain variable (V_L) domain REI and interleukin-1β (IL-1β). The goal of the Lee *et al.* paper was to see if they could insert the RGD sequence into the two specific "presentation scaffolds" and obtain binding to the platelet receptor. The RGD sequence motif participates in the interaction of a number of proteins with cell surface receptors called integrins. However, even though a number of proteins contain the RGD motif, only a small percentage of them are known to bind to the platelet receptor that the authors were studying ($\alpha IIb\beta_3$). (*See* p. 745, col.2) Further, Lee et al. found, however, that when a loop of REI V_L (an immunoglobulin domain) was replaced with an RGD sequence, the RGD sequence lost its biological function (page 751, Table I). Despite their use of sophisticated molecular modeling methods, the interleukin-1β (IL-1β)-RGD hybrid protein was inactive, demonstrating the difficulty in designing a biologically active loop (page 751, Table I).

Lee *et al.* only mention fibronectin at two places in the paper. First, on p. 745, where it is included in a list of natural ligands for the platelet receptor $\alpha IIb\beta_3$ that is being studied in the paper. Thus, fibronectin is being mentioned not as a potential scaffold, but as a potential ligand for the $\alpha IIb\beta_3$ integrin being studied in Lee *et al.* The second time fibronectin is mentioned is on p. 753, where the authors state that a number of other adhesion proteins contain the RGD motif, including the last loop of a fibronectin type 3 domain. Nowhere in Lee *et al.* is it mentioned that fibronectin can be modified as a "presentation scaffold," even though Lee *et al.* clearly were aware of the molecule. Therefore, the claims are not obvious over Lee *et al.*

The Federal Circuit in *In re Sang Su Lee*, Case No. 00-1158 (Fed. Cir. January 18, 2002)has recently stated the following:

> The factual inquiry whether to combine references must be thorough and searching. *Id.* It must be based on objective evidence of record. This precedent has been reinforced in myriad decisions, and cannot be dispensed with. *See, e.g., Brown & Williamson Tobacco Corp. v. Philip Morris Inc.*, 229 F.3d 1120, 1124-25, 56 USPQ2d 1456, 1459 (Fed. Cir. 2000) ("a showing of a suggestion, teaching, or motivation to combine the prior art references is an 'essential component of an obviousness holding'") (*quoting C.R. Bard, Inc., v. M3 Systems, Inc.*, 157 F.3d 1340, 1352, 48 USPQ2d 1225, 1232 (Fed. Cir. 1998)); *In re Dembiczak*, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999) ("Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references."); *In re Dance*, 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637 (Fed. Cir. 1998) (there must be some motivation, suggestion, or teaching of the desirability of making the specific combination that was made by the applicant); *In re Fine*, 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988) ("'teachings of references can be combined only if there is some suggestion or incentive to do so.'") (emphasis in original) (*quoting ACS Hosp. Sys., Inc. v. Montefiore Hosp.*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)).

It appears as if the Examiner is employing hindsight to arrive at Applicant's invention by picking and choosing from the disclosures in the prior art. The Examiner is reminded that it is impermissible to use Applicant's specification as a template to arrive at the conclusion that the claimed invention is obvious. *In re Fritsch*, 23 U.S.P.Q.2d 1780, 1782 (Fed. Cir. 1992). To render an invention obvious, the combination of the cited art must teach or suggest the claimed invention and provide a reasonable expectation of success in preparing the claimed invention. *In*

re Vaeck, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991); *In re O'Farrell*, 853 F.2d 894, 7 U.S.P.Q.2d 1673 (Fed. Cir. 1988).

The cited art does not provide a suggestion or motivation to combine Main *et al.* with Lee *et al.* As discussed above, Main *et al.* discloses the three-dimensional conformation of fibronectin, but does not discuss the possibility of structurally modifying the molecule by altering the amino acid sequence of a loop region. It only discusses the native form of the protein. Lee *et al.* discuss the modification of the immunoglobulin light chain variable domain REI and interleukin-1β. It does not teach or suggest the modification of fibronectin. Thus, even when combined, these references do not meet all three requirements for *prima facie* obviousness.

First, the references do not teach all the elements of the claimed invention, namely a fibronectin monobody with a varied loop region. Main *et al.* only discusses native fibronectin, and Lee *et al.* only discuss modifying two proteins, neither of which is fibronectin. Neither reference suggest modifying an FN3 loop region. Second, since there was no teaching of modifying FN3 loop regions, there could be no expectation of success. Third, there is no suggestion or incentive in the prior art itself that would have motivated a skilled worker to combine these two references, or to modify a reference. There is no such motivation or suggestion in either of the references. Again, Main *et al.* is directed to the conformational flexibility of a native protein so that the protein can modulate as a consequence of events within cells, and Lee *et al.* is directed to modification of two proteins to determine RGD-binding affinity. Nowhere in Lee *et al.* is it suggested that fibronectin should be similarly modified. It should be noted that Lee *et al.* had very specific criteria for the selection of their "presentation scaffolds" (see p. 750, Discussion).

Even if the examiner argues that the prior art provided a motivation to combine the references, Applicant respectfully asserts that there would not have been a reasonable expectation of success that the variation of an FN3 monobody loop region could be made with the claimed property of binding to an SBP. Lee *et al.* discuss two non-fibronectin proteins that were modified to have an RGD sequence. Their results indicated that the proteins did not consistently bind to its ligand (see Table 1). Therefore, even if one would have been motivated to attempt to

modify an FN3 monobody, there would not have been a reasonable expectation of success. There would only have been an invitation to experiment, which is not the legal standard.

It should be remembered that in the present invention the native RGD sequence is modified and/or replaced, whereas the focus of the Lee *et al.* reference was to put RGD binding sequences into the two scaffolds. Lee et al. focus on the problem of attempting to present the RGD binding sequence so that it will bind to its ligand, rather than on the problem of attempting to identify a scaffold sequence that could effectively present a variety of different binding sequences. Main *et al.* discussed RGD regions, but in the context of attempting to understand the conformational changes that could occur and still have the RGD motif bind to its ligand. Main *et al.* did not make any kind of sequence modification to the RGD, or any other part of the amino acid sequence. Thus, even though the present invention and the two cited references discuss binding regions, they do not teach the claimed invention.

Moreover, the examiner states on page 7 of the Office Action mailed August 18, 2000:

> As stated in Helms *et al.* "It is generally believed that loop regions in globular proteins, and particularly hypervariable loops in immunoglobulins, can accommodate a wide variety of sequence changes without jeopardizing protein structure or stability. We show here, however, that novel sequences introduced within complementarity determining regions (CDRs) 1 and 3 of the immunoglobulin variable domain REI VL can significantly diminish the stability of the native state of this protein" (see abstract and entire document). As stated in the specification of the Fn3 monobody "has a fold similar to that of immunoglobulin domains" (see page 18, lines 20-21) and as such one skilled in the art would reasonably conclude from Helms et al that not every loop replacement will result in a correctly folded protein wherein the loop region would bind to a specific binding partner. (emphasis added)

In particular, it was not obvious whether loops other than the FG loop (that contains the functional RGD sequence in the 10th Fn3 domain) could accommodate novel amino acid sequences. Therefore, it appears that the examiner agrees that, although Lee *et al.* and Main *et al.* teach that FN3 is homologous to an immunoglobulin domain and that it may be possible to use a presentation scaffold to display a functional peptide, one skilled in the art would reasonably conclude from Helms *et al.* that there was some uncertainty whether or not loop replacements could be made that would result in a varied loop region that can bind to a specific binding partner.

It has been demonstrated that the disulfide bond in the immunoglobulin domain is critical in maintaining its structure and stability (Goto and Hamaguchi, (1979) the role of the intrachain disulfide bond in the conformation and stability of the constant fragment of the immunoglobulin light chain, *J. Biochem.* 86:1433-1441). It is well known that disulfide bonds can stabilize a particular protein conformation by linking two cysteines that are distant along the polypeptide. Although the extensive experimental data on immunoglobulins have demonstrated that the immunoglobuin scaffold can accommodate numerous loop sequences, these immunoglobulin domains were stabilized by the critical disulfide bond. In contrast, the Fn3 scaffold does not contain disulfide bonds. The lack of stabilizing disulfide bonds makes it difficult to extrapolate the knowledge on immunoglobulins to the Fn3 scaffold and also makes it harder to predict the effects of loop mutations on the structure and stability of Fn3. Thus, one skilled in the art could not predict, without extensive experimentation, whether the Fn3 scaffold can be used as a "presentation scaffold."

Thus, it was not obvious *a priori* that the present inventor would be successful in making the claimed functional monobody until he actually performed the experiments. Applicant submits that it would require the impermissible application of hindsight to arrive at the claimed subject matter given the cited art. Such a use of hindsight is improper when resolving the question of obviousness. The direction must be provided by the art, not by the disclosure of the present specification.

Further, a number of research groups have sought ligands specific for various proteins. The work described by Twan van den Beucken *et al.* in the *Journal of Molecular Biology* (2001) **310**, 591-601 is illustrative. In this work, the authors state that "ligands specific for B7.1 (CD80) and B7.2 (CD86) have applications in disease indications that require inhibition of T-cell activity. As we observed significant sequence and structural similarity between the B7-binding ligand, cytotoxic T-lymphocyte associated protein-4 (CTLA-4), and antibody variable light chain domains (VLs), we have explored the possibilities of making novel B7 binding molecules based on single VL domains." (p. 591, beginning of the Abstract)

The investigators in the van den Beucken *et al.* paper described their rationale for what molecule to use that would bind to B7. Their discussion illustrates that there were not a large

number of protein scaffolds that would perform the desired function. In particular, they stated that "[i]n order to find protein frameworks with a similar structure onto which we could potentially graft functional fragments from CTLA-4, we performed a BLAST protein sequence-similarity search with human CTLA-4. All protein database coordinates for all VL chains were retrieved and the sequence alignment was repeated based on their three-dimensional structural alignment by fitting the main-chain atoms of the Ig fold β-strand onto structurally equivalent CTLA-4 atoms (data not shown)." ("Results" p. 592, col. 2) The scientists expected that it would be necessary to use a framework similar to that of CTLA-4 to correctly display the "functional fragments." They continued by stating that the "conclusion of this analysis was that despite the generally low degree of amino acid sequence similarity and the enormous conformational variability of the loops, the beta-sheets forming the framework were remarkably similar between most VL domains and CTLA-4. From this we reasoned that it should be possible to take one of these VL domains and graft on the CDR-like loops from CTLA-4 at structurally compatible sites." Were other frameworks obvious, it is unclear why they would have gone to this trouble.

Initially this seemed like a good line of reasoning in order to produce a B7 binder. This, however, was not the result. The authors went on to state that a "chimeric VL/CTLA-4 gene was constructed and cloned for expression and display. Low levels of soluble protein product were detected by Western blotting (Figure 1(c), lane 3) which suggested that this molecule was folded and could be secreted from *Escherichia coli*. However, no binding of the phage-displayed or soluble protein to the natural ligands B7.1 and B7.2 was observed (data not shown)." (page 592 end to page 593 top, emphasis added)

So, even using a framework that could reasonably be considered optimal, their efforts were not successful. Thus it took even these highly skilled practitioners in the art multiple attempts before they could generate scaffolds that could be used to display "CDR-like" loops and result in the B7 ligand binders that they sought. Of course, the authors were eventually successful, but this was only after preparing diverse libraries of VL frameworks and further applying *in vitro* mutagenesis. Negative results, such as those above, are not typically reported in the literature, except in the context of a subsequent success. It is possible, however, to see the

outlines of a significant problem in the art, for which there was no obvious solution, by looking at the obstacles that some very highly skilled practitioners had to overcome in order to achieve what the present invention provides so plainly and elegantly.

Another example of this is seen in the work of Desiderio *et al.* entitled, "A Semi-synthetic Repertoire of Intrinsically Stable Antibody Fragments Derived from a Single-framework Scaffold" (*Journal of Molecular Biology* (2001) 310, 603-615). This group, in the first sentence of their Abstract, p. 603, reports, "the design, construction and use of an antibody bacteriophage display library built on the scaffold of a single-chain variable fragment (scFv) previously proven to be functionally expressed in the reducing environment of both bacterial and plant cytoplasm and endowed with intrinsic high thermodynamic stability."

These authors comment (p. 604, col. 1) that "the use of phage display does not always ensure a selection of molecules endowed with excellent folding properties, high yields or stability. These properties are especially desirable when antibody fragments must be expressed in cellular environments that are incompatible with disulphide bond formation, such as in the reducing conditions of the cytoplasm. There is great interest in the ability to express functional intracellular antibodies (intrabodies) in this compartment for their potential immunotherapeutic use." Thus, numerous scaffolds with such properties were not common or obvious. The insight of their use of this particular antibody (a single-chain Fv fragment here) is useful, interesting, and highly publishable because obvious alternatives did not exist.

The Desiderio *et al.* authors took pains to use to antibodies as their scaffold. In fact, the scaffold that they used for this application was first published in 1993. They used an antibody scaffold because skilled practitioners knew that antibodies could provide a scaffold on which to make modifications and libraries of modifications. If it were obvious to use other scaffolds, and obvious that such scaffolds would work, there would have been little point to what they did here.

In their Discussion section on p.609 (col. 1), the Desiderio *et al.* scientists state, "Therefore, for applications that require a specific interference to cytoplasmic-related functions, it is fundamental to search for antibodies that can fold in the absence of disulphide bonds." A "search" would not be necessary if such scaffolds were "obvious." Further, their search would not have been limited to "antibodies" if it was obvious that other proteins would also work.

It should be noted that the claimed monobody scaffolds fully comply with the requirements stated by these authors. Monobodies do not have disulfide bonds. They readily fold and exhibit stabilities at temperatures of up to about 70° C or more, in some cases. They are soluble and can be produced at high levels in *E. coli.* In addition, the Desiderio *et al.* molecules, should they have retained any of the non-human character of their origin, would be distinctly inferior to the claimed human fibronectin-derived scaffold, because of their likely antigenicity in humans.

Finally, if it were obvious that other proteins could provide useful scaffolds, then certainly the first one that anyone would want to try would be a protein designed by nature to have a variable portion *in vivo* for the exact purpose of binding to and displaying small peptides, such as the T-cell receptor. In fact, numerous groups have worked with this molecule with the intent of harnessing it as a scaffold. The history of these efforts is best summarized by Shusta, *et al.* in their paper entitled, "Directed evolution of a stable scaffold for T-cell engineering" (Nature Biotechnology (2000) **18**, 754-759).

In the opening paragraph of their paper they state that although the diversity of TCRs is similar to that of antibodies, soluble TCRs have not yet been exploited in immunotherapeutical strategies with the potential to provide highly antigen-specific immunosuppression. They continue by stating that "such applications have not been realized for various reasons. Affinities of TCR/pMHC interactions are quite low (micromolar Kd), necessitating unfeasibly high concentrations of soluble TCR for targeting. Many TCRs have low solubility and a high propensity to aggregate, which is improve somewhat by fusion of the TCR variable regions to thioredoxin or antibody constant regions. Stability of soluble TCRs are low compared to antibodies, and recombinant production yields are low and variable."

The authors continue by stating the properties that would be desirable. Their paper further reports their own temperature-based screening system that allowed them to arrive at a relatively stable, soluble, single-chain T-cell receptor scaffold. Their methodology and the product produced were certainly not obvious. One might object that this is just an isolated case of the TCR being a problem. If this is the case, then there still was no guidance for what other

proteins would be useful as scaffolds without such an arduous process of experimentation and testing.

One of the interesting conclusions of the Shusta *et al.* authors was that there is a relationship between stability and improved display. They state in their final paragraph on page 758, "We had previously observed that well-displayed mutant scTCRs are more stable. In the present work, this connection has been strengthened by the observation that selection for increased stability in turn results in improved display (Fig. 1D,F). These findings suggest that in the case of the scTCR, the efficiency of the complex kinetic process of in vivo protein folding can be predicted with a single thermodynamic parameter, the in vitro stability of the protein (and vice versa). The robustness of this correlation gives confidence that this approach may be of general utility for obtaining stable scaffolds in other areas of protein engineering." (emphasis added)

It is evident from the literature that, rather than the present scaffolds being obvious, they instead are quite non-obvious and require extensive experimentation (that is not always successful) in order to be made. If the examiner does not find these three examples persuasive, as they were not directed to fibronectin, but instead to antibody-type domains, the examiner is reminded that one of the two proteins that Lee *et al.* worked with was an immunoglobulin domain (immunoglobulin V_L domain REI). Further, the examiner himself heavily relied on the similarity of FN3 and immunoglobulins in his "obviousness" rejection. At page 12 of the initial Office Action date September 18, 2000, the examiner stated:

> "One of ordinary skill in the art would have been motivated and would have had a reasonable expectation of success to have used the fibronectin type III domain as taught by Main *et al.* as a scaffold for replacement of amino acid residues as taught by Lee *et al.* because Main *et al.* teach that the fibronectin type III topology is similar to that if immunoglobulins and functional loops can be build onto a structural framework to be able to perform a wide range of functions."

Therefore, these references are as applicable to the present invention as Lee *et al.*

In conclusion, Applicants assert that the examiner has not presented a *prima facie* case of obviousness as there was no teaching or motivation in the art to combine the references, and because even if the references are combined, they do not teach all the features of the claims. In the alternative, Applicants assert that even if, *arguendo*, the Examiner has presented a proper

rejection under 35 U.S.C. § 103(a), one would not have had a reasonable expectation of success of generating the claimed invention. Accordingly, Applicant respectfully requests withdrawal of the rejection under 35 U.S.C. § 103(a).

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6961) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

SHOHEI KOIDE

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Date 15 February 2002 By _____
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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Box RCE, Commissioner of Patents, Washington, D.C. 20231, on this 15th day of February, 2002.

Candis B. Buending
Name

_____
Signature